University of São Paulo
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Introgression of natural genetic variation affecting trichome development and terpenes biosynthesis to obtain insect-resistant tomatoes

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Thesis presented to obtain the degree of Doctor in Science. Area: Plant Physiology and Biochemistry

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RESUMO

Introgressão de variações genéticas naturais que afetam o desenvolvimento de tricomas e a biossíntese de terpenos para obtenção de tomates resistentes a insetos

Para lidar com insetos pragas, as espécies selvagens do tomateiro produzem uma variedade de compostos de defesas em seus tricomas glandulares do tipo-VI. Por outro lado, embora as espécies cultivadas (*Solanum lycopersicum* L.) também apresentem tricomas do tipo-VI, a glândula do tomate cultivado é muito menor, contendo principalmente monoterpenos e baixos níveis de sesquiterpenos derivados do citosol, que não têm efeito aparente contra os insetos pragas. No presente trabalho, realizamos cruzamentos entre a espécie selvagem *S. habrochaites* e a cultivar de tomate Micro-Tom (MT), afim de se criar linhas de introgressão (ILs). Transferimos com sucesso a via do sesquiterpeno derivado do plastídeo de *S. habrochaites* para tricomas do tipo-VI do tomate cultivado (cv. Micro-Tom). Os tricomas da linhagem introgredida denominada MT-*Sesquiterpeno-sintase 2* (MT-*Sst2*) apresentaram concentrações ainda maiore de α-santaleno, β-bergamoteno e α-bergamoteno em comparação com os tricomas glandulares do tipo-VI da espécie selvagem. Surpreendentemente, a presença de grandes quantidades de sesquiterpenos derivados do plastídio ainda assim não foi suficiente para conferir resistência as pragas específicas de tomate no MT-*Sst2*. O perfil de sesquiterpeno de MT-*Sst2* e LA1777 revela compostos derivados de sesquiterpeno encontrados apenas na espécie selvagen, o que aponta para a necessidade de etapas adicionais para obteção de tomate resistente a insetos. Este trabalho abre caminho para o entendimento da morfologia e funcionalidade dos tricomas do tipo-VI e para a criação de tomates resistentes a insetos.

Palavras-chave: Tomate; Linhagem de introgressão; Tricoma glandular; Resistência à inseto; Sesquiterpeno.

ABSTRACT

Introgression of natural genetic variations affecting trichome development and terpenes biosynthesis to obtain insect-resistant tomatoes

To deal with insect pests the tomato wild relatives produce a variety of defensive compounds in their glandular trichomes type-VI. By contrast, although cultivated tomatoes (*Solanum lycopersicum* L.), also display type-VI trichomes, the gland in cultivated tomato is much smaller containing mainly monoterpenes and low levels of cytosolic-derived sesquiterpenes, which have no apparent effect against insect pests. In the present work, we carried out crosses between the wild species *S. habrochaites* and the tomato cultivar Micro-Tom (MT) in order to create introgression lines (ILs). We successfully transferred the plastid-derived sesquiterpene pathway from *S. habrochaites* to type-VI trichomes of the cultivated tomato (cv. Micro-Tom). The trichomes of the introgressed line named MT-*Sesquiterpene synthase 2* (MT-*Sst2*) showed even higher concentration of α-santalene, β-bergamotene, and α-bergamotene compared to the wild species type-VI glandular trichomes. Surprisingly, the presence of high amounts of plastid-derived sesquiterpenes was not sufficient to confer resistance to specific tomato pests in MT-*Sst2*. The sesquiterpene profile of MT-*Sst2* and LA1777 unveils sesquiterpene-derived compounds only found in the wild species, which point for additional steps necessary to obtain insect-resistant tomatoes. This work paves the way for both the understanding of the morphology and functionality of type-VI trichomes and for breeding insect resistant tomatoes.

Keywords: Tomato; Introgression line; Glandular trichomes; Insect resistance; Sesquiterpenes.

Terpenes are the most abundant and diverse class of compounds produced by plants with a wide variety of biological functions (Dudareva et al., 2013). They are produced by multiples of isoprene (C5) units. Isoprenoids are essential for plant growth and development. They participate as precursors for several components of photosynthesis, respiration, cell cycle control (Estévez et al., 2001) and plant hormones such as gibberellins, abscisic acid, brassinosteroids and strigolactones (Falara et al., 2011). Isoprenoids also play an important role in the interactions of plants with the environment, including defense against herbivorous insects and pollinators attraction (Dudareva et al., 2013).

In plants, all terpenes originate from two distinct metabolic pathways: the mevalonate (MVA) located in the cytosol and the 2-C-methyl-D-erythritol 4-phosphate (MEP) located in plastids. Both pathways produce and use isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to produce the isoprene building blocks used by terpene synthases (TPSs) to catalyze the formation of C10 monoterpenes, C15 sesquiterpenes or C20 diterpenes (Tholl, 2006).

In cultivated tomato (*Solanum lycopersicum*), sesquiterpene biosynthesis usually takes place in the cytosol through the MEV pathway. However, in glandular trichomes of the tomato-related wild species *S. habrochaites,* sesquiterpenes are also produced in the chloroplasts (Sallaud et al., 2009). The presence of plastid-derived sesquiterpenes in some wild species has been described to be responsible for the lower damage by insects, making these wild species naturally resistant to multiple pests such as lepidopterans (Eigenbrode et al., 1994; Eigenbrode et al., 1996), whiteflies (*Bemisia spp.*) (Bleeker et al., 2012) and also the herbivorous spider mites (Maluf et al., 2001).

Two independent loci have been associated with the biosynthesis of two different classes of sesquiterpenes in *S. habrochaites*. The *Sesquiterpene synthase 1* (*SsT1*) locus on chromosome 6 is responsible for the accumulation of class I cytosolic-derived sesquiterpenes. At this locus, the S. *lycopersicum TPS12* gene is associated with β-caryophyllene and α-humulene biosynthesis and *S. habrochaites TPS9* is associated with germacrene B production (Hoeven van der et al., 2000; Bleeker et al., 2011; Falara et al., 2011). The existence of a *S. lycopersicum TPS9 (SlTPS9)*, that makes germacrene C, was also reporter for the cv. VFNT Cherry (Colby et al., 1998), but it is worth noting that this cultivar has introgressions of the wild species *S. peruvianum* on chromosome 6. The second loci controlling sesquiterpenes is the *Sesquiterpene synthase 2* (*SsT2*) on chromosome 8. At this locus *S. habrochaites* encodes enzymes responsible for the accumulation of class II plastid-derived sesquiterpenes, including α-santalene, α-bergamotene, β-bergamotene and 7-epizingiberene. In cultivated tomato, a cluster of five functional TPS genes (*TPS18*, *TPS19*, *TPS20*, *TPS21*, and *TPS41*) is present in the equivalent locus on chromosome 8.

In addition, this same tomato chromosomal region also contains the *NERYL DIPHOSPHATE SYNTHASE 1* (*SlNDPS1*) gene, which codes for an enzyme catalyzing the formation of neryl diphosphate (NPP). The NPP is used by tomato TPS20 to synthesize β-phellandrene and several other monoterpenes in the chloroplasts (Falara et al., 2011; Matsuba et al., 2013).

The *SsT2* locus from *S. habrochaites* has a cluster of three functional TPS genes (*TPS18, TPS20* and *TPS45*) and the *CIS-FARNESYL DIPHOSPHATE SYNTHASE* (*zFPS*) gene, which is homologous to *SlNDPS1* (Matsuba et al., 2013). Both *zFPS* and *TPS45* contain putative chloroplast targeting sequences and are associated with the biosynthesis of class II sesquiterpenes in this organelle. The *zFPS* codes for a Z-prenyltransferase that catalyzes the synthesis of *Z-Z*-farnesyl diphosphate (*Z,Z*-FPP) from IPP and DMAPP. The *TPS45* gene encodes a Santalene and Bergamotene Synthase (SBS) that uses *Z,Z*-FPP as a substrate to produce class II sesquiterpenes (Sallaud et al., 2009; Matsuba et al., 2013). Both *zFPS* and *SBS* are expressed in type-VI trichomes (Sallaud et al., 2009) which are the most common and abundant glandular trichome found in several tomato-related species (Kang et al., 2010; Glas et al., 2012; Balcke et al., 2017). In cultivated tomato, type-VI trichomes contain a single basal cell connected to a short (~0.1 mm) unicellular stalk which is attached by an intermediate cell in the four-celled glandular head containing chloroplasts and other organelles (Bergau et al., 2015). In *S. habrochaites*, the type-VI glandular trichome has a distinct morphology with a longer stalk (~0.2 mm) and a round glandular head with a larger gland cavity (Besser et al., 2009).

In general, cultivated tomatoes are highly vulnerable to several arthropod pests, which include whiteflies, spider mites, and thrips. In heavy infestation, these pests can cause a reduction of plant vigor and yield which can lead to huge losses in productivity (Wakil et al., 2018). Consequently, to minimize the damage caused by pests, it has been necessary to apply high amount of pesticides (Silva et al., 2011). In this sense, an alternative to chemical pest control would be the use of commercial tomatoes carrying favorable genetic variations from tomato-related wild species. Herein, we investigated whether the introduction of the genetic pathway for class II plastid-derived sesquiterpenes into cultivated tomato could increase resistance to arthropod tomato pests. We show that the *Sst2* gene cluster that controls santalene and bergamotene in *S. habrochaites* LA1777 can be effectively transferred to cultivated tomato (cv. Micro-Tom) and function into its type-VI trichomes. We further demonstrated that, contrary to earlier works, the high production of "wild tomato sesquiterpenes" was not sufficient to confer resistance to the tomato-pests whitefly (*Bemisia tabaci*), spider mites (*Tetranychus urticae* and *T. evansi*) nor thrips (*Frankliniella occidentalis*). Conversely, the highly insect resistance presented by *S. habrochaites* LA1777 is likely to be due to sesquiterpene carboxylic acid derivatives that were detected in the wild species but not in the introgressed line.

This means that the full set of genes responsible for the conversion of the terpenes to derived carboxylic acids lie outside the metabolic cluster on chromosome 8. Surprisingly, the type-VI trichome internal storage-cavity size increased in the MT line harboring the *Sst2* gene cluster, which is probably a pleiotropic effect of the *S. habrochaites* genes leading to the high accumulation of santalene and bergamotene. Our results provide for the understanding of type-VI trichome morphology of *S. habrochaites* and pave the way for new discoveries of specific enzymes of sesquiterpene metabolism which will assist in the further breeding and pyramiding of all loci necessary to obtain insect-resistant tomatoes.

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